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DOI: <https://doi.org/10.2174/1871530315666150316120920>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-118090>

Journal Article

Accepted Version

Originally published at:

O'Connor, Tracy; Borsig, Lubor; Heikenwalder, Mathias (2015). CCL2-CCR2 signaling in disease pathogenesis. *Endocrine, Metabolic Immune Disorders Drug Targets*, 15(2):105-118.

DOI: <https://doi.org/10.2174/1871530315666150316120920>

CCL2-CCR2 signaling in disease pathogenesis

Tracy O'Connor^{1,†}, Lubor Borsig² and Mathias Heikenwalder^{1,†}

¹Institute of Virology, Technische Universität München/Helmholtz Zentrum München,
Munich, Germany

²Institute of Physiology, Zurich Center for Integrative Human Physiology, University of
Zurich, Zurich, Switzerland

Keywords: CCR2, CCL2, MCP-1, chemokine, cancer, metastasis, CNS, autoimmune disease

[†]Corresponding Authors:

Mathias Heikenwalder, PhD

Tracy O'Connor, PhD

Institute of Virology

Technische Universität München

Helmholtz Zentrum München

Schneckenburgerstraße 8

81675 Munich, Germany

Tel: +49 89 4140 7440

Fax: +49 89 4140 7444

Email: heikenwaelder@helmholtz-muenchen.de

Email: tracy.oconnor@helmholtz-muenchen.de

Summary

The role of chemokines and their receptors in controlling several physiological and pathological processes has only become evident in the last couple of years. From a sole function of chemo-attraction, our view on chemokine receptor activation has switched to the regulation of pleiotropic signaling pathways influencing numerous molecular and cellular processes. The large number of chemokines and receptors and hence possible combinations of chemokine-chemokine receptor interactions, as well as the expression profiles of chemokines and chemokine receptors within particular cell types, has contributed to the complexity of chemokine receptor signaling as we see it today. The chemokine CCL2 and its main chemokine receptor CCR2 have been implicated in the pathogenesis of several different disease processes, including vascular permeability and attraction of immune cells during metastasis, a number of different neurological disorders, autoimmune disease, obesity, and atherosclerosis. Here we review recent findings on the role of the CCL2-CCR2 axis in the regulation of these diseases. We believe that research has only gained a first glimpse of what chemokines can control and what the underlying mechanisms are. There is certainly more to be found that will - with high certainty - have strong implications for clinical applications in the near future.

On the role of CCL2-CCR2 signaling in cancer

Within the tumor, cancer cells co-exist with host cells, including bone marrow-derived, endothelial and stromal cells, and cancer progression depends on their communication. Tumor cells produce a number of soluble factors, such as growth factors, cytokines and chemokines that both promote tumor cell growth and also recruitment and/or activation of host cells within the tumor microenvironment. Altered chemokine-chemokine receptor axis affects both the composition and the function of cells within the tumor microenvironment and is linked to cancer progression [1-4]. While the contribution of inflammatory leukocytes, not only to cancer progression, but also to metastasis has been already recognized [4, 5], there is accumulating evidence that chemokines significantly contribute to metastasis through distinct mechanisms [1, 6]. Among the many chemokines associated with cancer progression, CCL2-CCR2 signaling has recently been identified as a major player in promoting tumorigenesis and metastasis [3, 4].

CCL2-CCR2 signaling in cancer: clinical evidence

Cancer progression and poor prognosis is linked to enhanced levels of CCL2 in number of cancers, including prostate, colon, breast, and cervical cancer [7-12]. High expression levels of CCL2 were a significant indicator of an early relapse in breast cancer patients [9]. In prostate cancer, CCL2 facilitates tumor growth via enhanced osteoclast and endothelial cell activity in bone marrow, thereby promoting bone metastasis [10]. Furthermore, CCL2 expression levels in prostate cancer correlated with the degree of tumor aggressiveness determined by Gleason score. Similarly, high CCL2 expression levels were linked to multiple enhanced liver metastases, and thereby, to poor prognosis for colorectal cancer patients [7]. The absence of CCL2 expression was associated with relapse-free survival in cervical cancers [11]. However, not only tumor cells, but also the surrounding stroma produced high levels of CCL2 as determined by immunohistochemical staining of a variety of tumors. These observations indicate that CCL2 stimulates the tumor microenvironment both in autocrine and paracrine fashion. In all analyzed cancer types, CCL2 expression was associated with increased infiltration of tumor-associated macrophages, which are known to be important for tumor progression, growth and angiogenesis.

CCL2-CCR2 signaling modulates the tumor microenvironment

It is currently accepted that tumor-derived chemokines actively shape the tumor microenvironment at primary or metastatic sites that are associated with the recruitment of leukocytes and activation of pro-inflammatory mediators [8, 13, 14]. In particular, CCL2 does not only contribute to recruitment of monocytic cells, but also exerts its own autocrine activity on the metastatic behavior of tumor cells. However, CCL2 may also suppress immune responses or even have antimetastatic activity, depending on the cellular context and the cancer type [15-18]. Current evidence shows that CCL2-mediated activation of CCR2⁺ endothelial cells directly contributes to tumor cell extravasation and metastasis [19]. Therefore, engagement of chemokine receptors on tumor cells can directly influence their behavior and invasive capabilities, and activation of CCR2 receptors on stromal cells and leukocytes can promote cancer progression.

Autocrine role of CCL2 on metastatic tumor cell behavior

Tumor-derived chemokines have been shown to directly affect tumor cells in an autocrine manner, including CCL2. CCL2-initiated signaling promotes tumor cell survival and metastasis of prostate cancer to the bone, and CCR2 overexpression in prostate cancer correlated with poor prognosis [3, 20]. Further support for a direct role of CCL2 on tumor cells was obtained by CCL2 treatment of PC-3 prostate cancer cells that protected cells from nutrient-induced autophagic death through activation of the PI3K/Akt/survivin pathway [21]. CCL2 treatment of PC-3 cells increased migration of the cells, which was associated with increased $\alpha\beta3$ integrin expression [22]. In breast cancer cells, increased CCL2 expression corresponded with up-regulated CCR2 expression and resulted in enhanced survival [23]. Accordingly, silencing of CCR2 in breast cancer cells significantly attenuated CCL2-driven cell migration and survival. In high-grade bladder cancer, cells expressing high levels of CCL2 promoted the migration and invasive capacity of these cells, when compared to low-grade bladder cancer cells expressing low CCL2 levels [24].

Anti-metastatic activity of the CCL2-CCR2 axis

Contrary to accumulating evidence that the CCL2-CCR2 axis promotes metastasis, there are several reports describing the opposite effect, resulting in inhibition of tumor progression and metastasis in a breast cancer model [17, 18]. Overexpression of CCL2 in 4T1 breast

cancer cells resulted in attenuation of metastasis; however, the mechanism remains to be clarified [17]. In another study, CCL2 had a pro-tumorigenic activity at primary sites, while it inhibited metastasis to the lungs in an orthotopic breast cancer model using 4T1 cells [18]. Down-regulation of CCL2 in tumor cells resulted in reduced primary tumor growth but accelerated metastasis. Further evaluations indicated that tumor-associated neutrophils may be differentially modulated by CCL2. Depletion of neutrophils showed no effect on the primary tumor growth, but resulted in enhanced lung metastasis. This study suggested that neutrophils have a protective role and prevent metastasis. The switch in the neutrophil phenotypes between these anti- and pro-tumorigenic phenotypes was shown to be regulated by tumor cell-derived TGF β [18, 25]. Nevertheless, the role of granulocytic cells/neutrophils (Ly6G⁺Ly6C⁺ cells) during cancer progression remains controversial [26]. Using a metastatic variant of 4T1 cells, depletion of granulocytic cells using anti-Gr1 or anti-Ly6G antibodies reduced circulating numbers of granulocytic cells resulting in attenuation of metastasis. Based on this opposite findings, further studies addressing different cancer models and cellular composition are required to fully understand the apparently ambivalent role of CCL2-CCR2 signaling in cancer progression.

CCL2 as a modulator of immune suppression in cancer?

Different tumors use different mechanisms to sustain tumor growth and promote metastatic dissemination. In a melanoma model, CCL2-dependent recruitment of monocytic CCR2⁺ myeloid-derived suppressor cells (MDSCs) contributed to immune escape and tumor growth [15]. Accordingly, depletion of CCR2⁺ MDSCs resulted in increased infiltration of activated cytotoxic CD8⁺ T-cells and reduced tumor growth. The use of anti-CCL2 antibody in a non-small-cell cancer model resulted in reduced tumor growth and attenuated lung metastasis [16]. Despite minor monocyte/macrophages alterations, their polarization status changed, which increased the presence of activated CD8⁺ cells.

CCL2 as a mediator of monocytic cell recruitment during metastasis

Leukocyte recruitment to sites of inflammation or metastasis is driven by a chemokine gradient which is formed by chemokine binding to glycosaminoglycans in the extracellular matrix and on the endothelium. Direct evidence for the role of CCL2-glycosaminoglycans interactions in recruitment of monocytic cells has been recently shown in a metastatic

model [27]. Suppression of proteoglycan versican expression led to attenuated metastasis. Furthermore, the lack of versican resulted in reduced presence of CCL2 and thereby reduced monocyte infiltration and metastasis. In several independent studies metastasis of breast and colon cancer cells to the lungs, the bone, and the liver of mice were shown to be dependent on CCL2 expression by the tumor cells [19, 28-30]. CCL2 overexpression in MDA-MB-231 human breast cancer cells promoted experimental metastasis to the lungs and the bone, which was significantly reduced with the application of CCL2-neutralizing antibodies [28]. Similarly, experimental metastasis of colon cancer cells (MC-38GFP) and Lewis lung carcinoma cells was found to be dependent on CCL2 expression by tumor cells [19, 30]. Specific down-regulation of CCL2 in both MC-38 and 3LL cells resulted in a significantly reduced metastatic capacity, indicating that tumor cell-derived chemokines are required for recruitment of monocytes. Recruitment of circulating monocytic cells to metastatic sites has been identified as a critical factor for breast, colon, and lung cancer dissemination [19, 29]. The population of monocytes has been defined as CD11b⁺, CCR2⁺ and Ly6C^{hi} positive cells that are recruited to metastasizing tumor cells and promote efficient tumor cell extravasation [19, 29]. The use of a transgenic mouse model deficient in CCR2 expression in myeloid cells (LysMCreCcr2^{fl/fl}) showed reduced metastasis, confirming the role of monocytic cells in metastasis [19]. Similarly, a distinct population of CCR2⁺/CD11b/Gr1^{mid} cells has been identified which promote experimental liver metastasis [30]. These studies provided evidence for a role of CCL2-driven recruitment of monocytic cells during tumor cell colonization of distant organs. Still, the exact mechanism of how these monocytic cells promote metastasis requires further investigation in the context of the metastatic microenvironment.

Glioma

Glioma cells secrete chemokines to attract microglia and macrophages, which, in turn, promote growth and migration of tumor cells [31]. Elevated CCL2 levels have been detected in human glioma samples [32, 33], and production of CCL2 by glioma cells can be stimulated by ATP and S100B [34, 35]. Blocking CCL2 function with a neutralizing antibody reduces microglia/macrophage infiltration and prolongs the survival of mice with gliomas [36], indicating that CCL2 secretion plays a key role in the recruitment of these cells during

tumorigenesis. Interestingly, CCR2 expression was found to emanate from tumor cells in addition to monocytes in a gliosarcoma model [37].

Stromal-derived CCL2 contributes to metastasis

The cross-talk between tumor cells and the surrounding stroma is critical for metastasis. Stromal-derived CCL2 has been shown to promote cancer progression. In a breast cancer model, cancer-associated fibroblasts produce CCL2 that regulates stromal-epithelial interactions [38]. In addition, increased CCL2 production has been achieved by specific inhibition of TGF β signaling in fibroblasts that resulted in increased recruitment of tumor-associated macrophages. Treatment with CCL2-neutralizing antibodies abrogated tumorigenesis and metastasis [38]. Recently, the analysis of stromal-derived CCL2 on tumor growth and metastasis has been evaluated in Ccl2-deficient mice (Ccl2^{-/-}) [39]. Although primary tumor growth upon injection of 4T1 breast cancer cells was not affected, lung metastasis was significantly attenuated in Ccl2^{-/-} mice compared to wt mice. Interestingly, lung metastasis could be rescued by wild-type bone marrow transplantation into lethally irradiated Ccl2^{-/-} mice. Since Ccl2^{-/-} bone marrow transplantation into wt mice did not affect metastasis, the authors concluded that stromal-derived CCL2 in the primary tumor promotes lungs metastasis. However, the exact mechanism remains to be defined.

Does CCL2 promote metastatic niche formation?

The involvement of the CCL2-CCR2 axis in the formation of a metastatic niche has been mostly studied in prostate cancer models studying bone metastasis [10, 12, 40]. Chemotaxis of prostate cancer cells is driven by CCL2, indicating the possible involvement of CCL2 in tissue specific migration [10, 40]. In addition, the secretion of parathyroid hormone-related protein by prostate cancer cells induced CCL2 production by osteoblasts in the bone microenvironment [12]. Enhanced levels of CCL2 in the bone stimulated osteoclast activation and bone resorption [20, 41]. Accordingly, tumor growth in the bone was significantly attenuated by CCL2-neutralizing antibody treatment [12]. When prostate cancer-bearing mice were treated with cyclophosphamide, induced expression of cytokines and chemokines, including CCL2, was detected [42]. This treatment also resulted in increased recruitment of myeloid cells to the metastatic sites and enhanced bone metastasis, which could be reduced by anti-CCL2 neutralizing antibody treatment. This study

1 indicated that cytotoxic therapy may contribute to bone metastasis through a transient
2 perturbation of the myeloid cell release.

3 ***Endothelial CCR2 expression facilitates tumor cell extravasation***

4 Attenuation of lung metastasis was observed in *Ccr2*^{-/-} mice compared to wild-type mice
5 using an experimental metastasis model. Efficient tumor cell extravasation has been shown
6 to be dependent on CCR2 engagement on endothelial cells in the lung microvasculature [19]
7 (Figure 1). The lack of stromal CCR2 expression led to a reduced lung tumor burden
8 compared to control mice. Mice expressing CCR2 exclusively on endothelial cells under the
9 Tie2 promoter showed only a small attenuation of metastasis compared to wild-type
10 controls but had significantly more metastatic foci compared to *Ccr2*^{-/-} mice. Importantly,
11 increased vascular permeability and subsequent tumor cell extravasation was shown to be
12 dependent on endothelial CCR2 expression [19]. Tumor cell transmigration through CCR2-
13 deficient endothelial cells was significantly reduced, demonstrating the indispensable role
14 for endothelial CCR2 in facilitating tumor cell transmigration and metastasis. These findings
15 are in agreement with a previous observation that CCR2-dependent activation of vascular
16 cells is required for increased vascular permeability and efficient leukocyte extravasation in
17 brain inflammation models [43].

18 Further analysis of the signaling mechanism in endothelial cells *in vivo* revealed that
19 inhibition of JAK2, the direct downstream mediator of CCR2, inhibited tumor cell
20 transmigration *in vitro* and diminished the induction of lung permeability, consequently
21 attenuating metastasis [19] (Figure 1). Downstream signaling of JAK2 has been identified to
22 be mediated through Stat5 and p38MAPK. Inhibition of both pathways resulted in reduced
23 lung vascular permeability and tumor cell extravasation. This was in agreement with
24 previous findings where transmigrating tumor cells activated endothelial cells with
25 upregulated p38MAPK pathway [44]. Yet, the chemokine-mediated activation of the
26 p38MAPK pathway in endothelial cells has not been previously reported. Similarly, the role
27 of Stat5 activation in promoting metastasis remains to be defined.

28 Taken together, the finding that endothelial CCR2 determines the efficacy of tumor cell
29 extravasation indicates that tumor-derived CCL2 induces vascular permeability which is
30 followed by the CCR2-dependent recruitment of monocytic cells enabling efficient

metastasis (Figure 1). Whether endothelial CCR2 expression is also a determining factor in tissues other than lungs remains to be determined. However, the observed CCR2 expression in brain microvascular endothelial cells associated with enhanced vascular permeability suggests that the CCR2-mediated vascular permeability may be a more common mechanism for metastasis than currently recognized.

CCL2-CCR2 signaling in the nervous system

In addition to its roles in the periphery, CCL2-CCR2 signaling also has important functions in the nervous system. Microglia, the resident immune cells of the central nervous system (CNS), are phylogenetically related to monocytes and thus also express CCR2. Typically, CCL2 secreted by activated astrocytes, the primary glial cell in the CNS, is thought to attract microglia to sites of neuronal infection or injury, where they phagocytose microbes or cellular debris [45-52]. However, CCL2 can also be produced by microglia/macrophages themselves, endothelial cells [49, 53-55] and neurons [56-58] under both basal and neuroinflammatory conditions. Monocytes from the periphery can also migrate to the CNS in response to neuroinflammation [49, 59, 60]. In this case, not only monocytes, but also CCR2-expressing brain microvascular endothelial cells (BMECs) respond to CCL2, which enhances the permeability of the blood-brain barrier (BBB) *via* RhoA signaling and reorganization of the actin cytoskeleton [43, 61-64]. Interestingly, neural progenitor cells (NPCs) are also attracted to sites of neuronal damage, and CCL2-CCR2 signaling seems to be important for this process, as well [65]. CCL2-CCR2-dependent migration of NPCs in the CNS has been reported during a number of brain conditions, including ischemia and stroke [66, 67], glial tumors [68], striatal cell loss [69], and epilepsy [70]. Neuronal [58, 71-73] and astrocytic [74, 75] CCR2 expression has also been reported. Activation of astrocytic CCR2 enhances their survival *via* NF- κ B and Akt signaling pathways [76] and promotes the production of neurotrophic factors [77].

Ischemia & Stroke

BBB disruption and immune cell infiltration of the brain parenchyma occur during cerebral infarcts and ischemia. The resulting distinct types of pro-inflammatory responses, including upregulated CCL2 levels in humans [49, 78-82] and rodents [49, 83, 84], can contribute to

neuronal damage. CCL2-CCR2 signaling seems to be an important mediator of this damaging inflammatory response, since *Ccl2*^{-/-} mice exhibit reduced lesion size after middle cerebral artery occlusion (MCAO; [85]), CCL2 gene disruption in rats reduced infarct volumes after induction of focal cerebral ischemia [86], and *Ccr2*^{-/-} mice experience reduced infarct size, BBB permeability, and brain edema, as well as improved motor function after experimental induction of focal transient cerebral ischemia or intracerebral hemorrhage (ICH) compared to wild-type mice [87, 88]. Conversely, CCL2 overexpression exacerbates ischemic brain injury in mice [89]. CCL2 is known to be important for recruiting circulating pro-inflammatory monocytes in the blood during ischemia [90, 91]. Recently, these peripheral monocytes have been identified as the primary damage-mediating cell, as opposed to resident microglia, since chimeric mice expressing wild-type CCR2 in the CNS and a *Ccr2*^{-/-} hematopoietic compartment also experienced improved motor function after ICH compared to wild-type mice [88]. In addition to its role in leukocyte recruitment, CCL2 may also promote BBB breakdown during stroke [92]. Moreover, endothelial cells near ischemic lesions also produce CCL2 in order to attract peripheral macrophages [93].

Neurodegeneration

Neurodegeneration is associated with a massive neuroimmune response marked by astro- and microgliosis and often also by the accumulation of aberrant protein aggregates in or around affected brain cells (e.g. amyloid β [A β] and tau in Alzheimer's disease [AD], superoxide dismutase 1 [SOD1] and TAR DNA-binding protein 43 [TDP-43] in amyotrophic lateral sclerosis [ALS], α -synuclein in Parkinson's disease [PD], and PrP^{Sc} in prion disease). Accordingly, CCL2 upregulation has been identified in a number of neurodegenerative disorders, including ALS [94-101], PD [102], and AD [103-108], as well as rodent models of AD [109, 110], PD [111, 112], ALS [113], and prion disease [114]. Genetic deletion of CCR2 or CCL2 in mouse models of AD-related amyloid plaque pathology enhances A β deposition, exacerbates senile plaque pathology, and accelerates cognitive decline [115-117], supporting the idea that CCL2-CCR2 signaling may be beneficial in AD, presumably via enhanced microglial recruitment and A β clearance. However, CCL2 overexpression also enhanced A β deposition and accelerated cognitive decline in similar transgenic models [118, 119]. Moreover, adenoviral delivery of dominant-negative CCL2 to the CNS of AD

transgenics actually suppressed A β accumulation and improved cognitive performance in another study [119]. Therefore, CCL2-CCR2 signaling appears to have both positive and negative influences during the course of AD pathogenesis. The authors of the latter studies have speculated that enhanced microglial recruitment upregulates local levels of apolipoprotein E (ApoE), which is known to influence the fibrillization of A β [120]. Thus, upregulated ApoE may also reduce the solubility of extracellular A β and hence its ability to be cleared. The benefit of enhanced CCR2-CCL2 signaling in other neurodegenerative disorders is similarly unclear. For example, no difference was found in the amount of striatal cell damage induced in wild-type versus *Ccl2*^{-/-} mice in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD [112], and neither deletion of CCL2 nor CCR2 accelerated the disease course in prion-infected mice [114, 121].

Excitotoxic and mechanical injury

Neurons can be damaged by the release of excitatory neurotransmitters, such as glutamate, from dying or rapidly firing neighboring neurons during e.g. stroke, neurodegeneration, trauma, or seizure. Elevated CCL2 secretion and/or CCR2 expression occurs during acute excitotoxic injury in rat brain [122-124], NMDA- or glutamate treatment in rat brain slices [125, 126], status epilepticus in rats [127], and traumatic brain injury (TBI) in mouse astrocytes [48, 128]. CCL2-CCR2 signaling seems to exacerbate excitotoxicity *in vivo*, since CCL2 neutralizing antibodies ameliorate excitotoxic damage or TBI in rat brain [123, 129]. However, CCL2-CCR2 signaling may also confer some benefit, since addition of CCL2 protected neurons from NMDA-induced damage *in vitro* [130, 131]. Interestingly, CCL2-CCR2 signaling is implicated not only in damage, but also in neuropathic pain associated with enhanced excitatory transmission. Elevated CCR2 expression has been reported in *ex vivo* [132] and *in vivo* [133] models of neuropathic pain, and *Ccr2*^{-/-} mice are resistant to neuropathic pain [134].

Neurological complications of HIV infections

During HIV infections, macrophages/microglia in the brain can become infected and/or activated, causing neurological complications, such as encephalitis and cognitive impairment. CCL2-CCR2 signaling has been implicated in HIV-associated glial activation and HIV neuroinvasion. CCL2 levels correlated with degree of brain injury [135] and CSF CCL2

levels correlated with CSF viral titers and severity of encephalitis in HIV-infected patients [136, 137]. HIV transactivating (Tat) protein induces CCL2 production [138] in astrocytes [139] and microglia [140], the secretion of which seems to protect neurons and astrocytes from Tat-induced toxicity [141]. Moreover, stimulation of CCR2 on macrophages by astrocyte-derived CCL2 suppresses interferon (IFN) α signaling while stimulating IFN β [142]. CCL2-CCR2 signaling may also mediate HIV entry into the CNS, as HIV-infected peripheral macrophages upregulate CCR2 [143, 144] and CCL2 [145, 146] and exhibit an enhanced responsiveness to CCL2 and ability to cross the BBB [143, 144].

CCL2-CCR2 signaling in multiple sclerosis and other autoimmune diseases

Multiple Sclerosis

CCL2-CCR2 signaling has also been implicated in the pathogenesis of autoimmune diseases, especially multiple sclerosis (MS), in which nerve damage is caused by an inappropriate T-cell response generated against self-antigens in the myelin sheath, which surrounds and electrically insulates neuronal axons. CCL2 and/or CCR2 expression is upregulated in lesions, blood, and CSF from MS patients [147-152] and in animal models of MS, experimental autoimmune encephalomyelitis (EAE), in rats [53, 153] and mice [45, 154-156]. *Ccr2*^{-/-} and *Ccl2*^{-/-} mice are at least partially resistant to EAE [157-160], CCL2 blocking antibody prevents EAE relapse in wild-type mice [155, 156], and DNA vaccination against CCL2 confers resistance to EAE [161], indicating that CCL2-CCR2 signaling is detrimental during MS pathogenesis. Mononuclear cells seems to be the main CCL2-responsive cell in EAE, since *Ccr2*^{-/-} mice adoptively transferred with primed T-cells from wild-type mice were also resistant to disease [157]. Nevertheless, CCL2-CCR2 signaling is somehow involved in maintaining the T-cell response, as both T-cell and monocyte infiltration was reduced in *Ccr2*^{-/-} mice [157-159]. Moreover, CCR2⁺CCR5⁺ T-cells were found to be selectively enriched in CSF from relapsing MS patients [162]; therefore, CCR2 expression in T-cells may also play a role in human MS. Stromal CCL2 expression is required for efficient EAE induction [163], and recently, it has been shown that astrocytes are a key source of CCL2, since astrocyte-specific depletion of CCL2 reduced clinical severity, axonal loss, and monocyte infiltration during EAE [164, 165]. However, CCL2 produced by BMECs may also be important for disease induction, since endothelium-specific CCL2 depletion in mice delays EAE onset [165].

Other autoimmune disorders

In addition to MS, CCL2 protein is also upregulated in the blood from patients with rheumatoid arthritis (RA; [166]). CCL2-CCR2 signaling appears to be protective in RA, as *Ccr2*^{-/-} mice are more susceptible to experimentally-induced arthritis [167]. Human polymorphisms in CCL2 have also been found to be associated with lupus erythematosus [168]. In addition, genetic deletion or pharmacological inhibition of CCR2 conferred disease resistance in a murine model of Guillain-Barré syndrome [169]. Finally, it was recently reported that loss of functional CCR2 reduced immune cell infiltration and ameliorated retinal damage in a mouse model of autoimmune uveitis [170].

CCL2-CCR2 signaling in metabolic syndrome and cardiovascular disease

Obesity

Obesity is associated with chronic, low-grade inflammation, including enhanced infiltration (from 10 – 15% up to 40 - 50%) of visceral adipose tissue by activated macrophages [171]. It is suspected that CCL2-CCR2 signaling could play a role in recruiting monocytes to adipose tissue, since adipocytes upregulate CCL2 expression during obesity [172], and CCL2 levels are elevated in adipose tissue or serum in mice on a high-fat diet [173, 174] and genetically obese mice [175]. However, studies investigating the effect of CCR2 deletion on adipose inflammation and insulin resistance in mouse models have yielded conflicting results [174-179]. Therefore, CCL2-CCR2 signaling in adipose tissue may be functionally redundant with other chemokine signaling pathways, e.g. CCR5 [180, 181].

Atherosclerosis

Atherosclerosis is the thickening of arterial walls and the formation of obstructive plaques due to the accumulation of platelets, leukocytes, and lipids in arteries. CCL2-CCR2 signaling is thought to play an exacerbating role in atherosclerosis, presumably *via* the recruitment of inflammatory monocytes to the site of atherosclerotic plaques [182]. Genetic deletion of CCL2 or CCR2 slows the disease course in animal models of atherosclerosis [183-185]. Moreover, blocking CCL2-CCR2 signaling pharmacologically also reduced the formation of atherosclerotic plaques [186, 187]. Hence, targeting CCL2-CCR2 signaling appears to be a

1 viable strategy for the treatment of atherosclerosis which is currently being pursued in
2 clinical trials.

4 **Future directions to exploit our knowledge of chemokines for therapy**

5 ***Anti-metastatic therapy***

6 As indicated above, several pathophysiological processes correlate with or are functionally
7 connected to deregulated CCL2/CCR2 expression. Several of these diseases might be
8 partially treatable with an efficient, targeted inhibition of CCR2 signaling. This also holds
9 true for exploiting our knowledge of CCR2 signaling for the treatment of tumors or
10 metastasis. To prevent or suppress the spread of already existing metastases is one of the
11 most important scientific, clinical and medical assignments in the last decade of cancer
12 research [13]. Unfortunately, efficient inhibition of metastasis by the targeting of tumor
13 cells or specific host cells is currently only achieved in very few cases. This is primarily due to
14 limited time windows of action and the complex interplay between various host cell types
15 and tumor cells and different types of cancer with distinct treatment responses. The
16 concept of pharmacological inactivation of chemokines or chemokine receptors in order to
17 reduce chemokine-dependent metastasis is under investigation and is not entirely novel [3,
18 188]. However, there are various obstacles that make inactivation of chemokines and their
19 receptors *in vivo* a challenge [4]:

- 20 - Inhibition of chemokine signaling may lead to compensatory effects resulting in
21 changes of chemokine profiles [4, 30]
- 22 - The interactome of chemokines and chemokine receptors is complex, with various
23 chemokines binding to one and the same receptor but also various chemokine
24 receptors recognizing the same ligand [4].
- 25 - Inactivation or inhibition of chemokine receptor-expressing cells may have severe
26 consequences, due to the wide range of cells expressing chemokine receptors,
27 although a time-defined treatment may be tolerated [4]. Therefore, specific

chemokine-chemokine receptor functions may be successful only within a specific time window.

Thus, one of the most important scientific challenges of the near future is to develop targeted therapies to suppress chemokine receptor signaling at specific sites of action (e.g. endothelial cells in specific organs). Moreover, there is an urgent need for novel tools (for example, small molecules, function-blocking antibodies) and additional mechanistic knowledge to successfully achieve a targeted and efficient inactivation of either chemokines or their receptors [4]. Despite several uncertainties, inhibition of CCL2 or CCR2 was reported to be beneficial in inhibiting metastasis of various cancer types such as breast, bladder, colorectal or prostate cancer in experimental models [19, 29, 30]. Therefore, clinical trials have been started, with the aim of assessing the safety and efficacy of inhibiting CCL2 or CCR2 in metastatic patients (<http://www.clinicaltrials.gov>: NCT00992186, NCT01015560) [4]. The first trial for the treatment of patients with metastatic castration-resistant prostate cancer applied the antibody 'CNTO 888,' which blocks CCL2 [4]. Surprisingly, CCL2 blockade was less effective - possibly due to compensatory mechanisms that led to an increase in CCL2 expression. In a second clinical trial (phase II), which is currently ongoing, the anti-CCR2 antibody 'MLN1202' is used for the treatment of patients with bone metastasis. Importantly, no anti-metastatic therapies exist. Therefore, additional criteria assessing efficacy will be required. Moreover, future studies will show which anti-CCR2 or anti-CCL2 blocking reagents can be used in the clinics. Moreover, it is very possible that additional chemokine-chemokine receptor pairs can be targeted for clinical evaluation for the treatment of metastatic patients.

Other disorders

In addition to cancer, CCR2/CCL2-targeted therapies are also being pursued as treatments for other disorders. For example, a CCR5/CCR2 inhibitor, cenicriviroc, is currently in Phase II clinical trials for the treatment of HIV-associated neurocognitive disorder, and a Phase II study has been completed with a CCR2 antagonist for the treatment of osteoarthritic knee pain. Finally, CCL2 neutralizing antibodies have been used in Phase I and Phase IIa clinical trials for the treatment of lupus erythematosus and diabetic nephropathy, respectively (www.clinicaltrials.gov; NCT01712061, NCT02128828, NCT00689273, NCT00976729). Phase

1 II clinical trials have also been completed investigating the effect of CCR2 blockade on serum
2 C-reactive protein levels in arthritic patients (www.clinicaltrials.gov; NCT00715169) and a
3 CCR2 antagonist on plasma glucose levels in patients with insulin resistance
4 (www.clinicaltrials.gov; NCT00699790).

5 **Conclusions**

6 In summary, breathtaking advance has been made in the last years in understanding the
7 underlying molecular and cellular mechanisms of CCL2-CCR2 signaling in health and disease.
8 Translation of this progress from basic research is still in the starting phase, although initial
9 clinical trials are running, currently launched, or are upcoming. Thus, it will take several
10 more years until larger patient cohorts will profit from the translation of basic research on
11 CCL2/CCR2 biology into the clinics.

13 **Acknowledgments**

14 LB was supported by Swiss National Foundation (310030-152901) and by EuroNanoMed2
15 2nd call; project NANODIATER. MH was supported by the Helmholtz foundation, a
16 Starting ERC grant (LiverCancerMechanism), the SFB transregio 36 (SFBTR36), the
17 Helmholtz alliance Preclinical cancer center (PCCC), the Foundation for Experimental
18 Biomedicine Zürich (Hofschneider foundation), and the Graduierten Kolleg GRK482.

Figures

Figure 1: Involvement of CCL2–CCR2-mediated signaling in tumor cell extravasation. (a)

Tumor cell-derived CCL2 recruits CCR2⁺ monocytes. Upon binding to endothelial CCR2, endothelial cells become activated and trigger various signaling pathways. (b) Triggering of endothelial CCR2 by CCL2 leads to phosphorylation of JAK2, subsequently activating various downstream signaling pathways. Signaling through Stat5 and p38MAPK pathways is important for tumor cell extravasation whereas Stat3, PI3K and Rac1 activation seems not to be involved. (c) Activation of endothelial cells via the CCL2–CCR2-axis leads to cytoskeletal retraction within endothelial cells resulting in induction of vascular permeability. Disruption of the endothelial layer and gap formation between endothelial cells then allows transmigration of tumor cells together with monocytes. However, the exact dynamics and kinetics of cell–cell interactions promoting transmigration remain to be determined.

Adapted from Borsig, et al., 2014 [4].

Figure 2: The role of CCL2-CCR2 signaling in the CNS. Damage or microbial infection in the

brain parenchyma triggers the release of CCL2 from astrocytes, microglia, and neurons. The release of CCL2 then induces recruitment of microglia, astrocytes, and neural progenitor cells (NPCs) to sites of damage or infection *via* activation of the CCR2 receptor. In addition, CCR2 activation on brain microvascular endothelial cells (BMECs) enhances blood-brain barrier permeability and facilitates the recruitment of CCR2-expressing inflammatory monocytes from the circulation into the brain parenchyma.

Figure 3: The role of chemokines in obesity. Under normal conditions (A) adipocytes

release chemokines, e.g. CCL2, CCL5 which leads to the recruitment of immune cells, such as macrophages (accounting for 10 – 15% of adipose tissue cellular content), from the circulation to adipose tissue. During obesity (B), the production of chemokines by adipocytes increases, leading to enhanced recruitment of immune cells to adipose tissue. In contrast to normal conditions, in obesity, macrophages can account for up to 40 – 50% of adipose tissue [171].

1 **Tables**

2 **Table 1**

Disorder	Proposed role of CCL2-CCR2 signaling	References
Progression of prostate, colon, breast, and cervical cancer	Macrophage infiltration	[7-12]
Prostate to bone metastasis	Osteoclast activation	[10]
Breast/ colon cancer metastasis	Inflammatory monocyte recruitment; altered vascular permeability	[19, 29]
Ischemia/Stroke	Microglia/ peripheral monocyte and NPC recruitment; BBB permeability	[66, 67, 85-88, 90-93]
Alzheimer's disease	A β clearance; A β fibrillization	[115-119]
Prion disease	Microglia recruitment; prion replication/ degradation	[114, 121]
Excitotoxicity	Microglia/monocyte and NPC recruitment	[70, 123, 130, 131]
Traumatic Brain Injury	Microglia/monocyte and NPC recruitment	[129]
Neuropathic pain	Sensitization of neurons to excitatory stimulation	[134]
HIV-associated neurological dysfunction	Microglia/ monocyte recruitment & activation; HIV neuroinvasion	[138-146]
Glioma	Microglia/monocyte and NPC recruitment	[36]
Multiple sclerosis	Inflammatory monocyte/ T-cell recruitment; BBB permeability	[155-161, 163-165]
Arthritis	Inhibition of autoantibody production	[167]
Guillain-Barré syndrome	Inflammatory monocyte recruitment	[169]

Autoimmune uveitis	Inflammatory monocyte/ T-cell recruitment	[170]
Atherosclerosis	Inflammatory monocyte recruitment	[183-187]
Obesity	Inflammatory monocyte recruitment	[174-179]

1 **Table I:** Disorders in which a role for CCL2-CCR2 signaling has been experimentally demonstrated.

2

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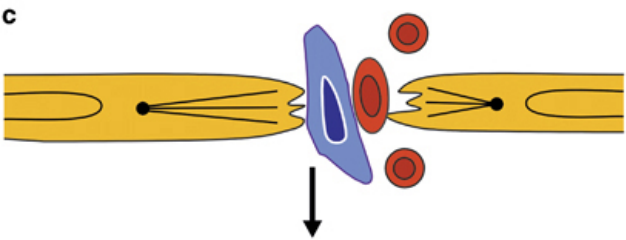
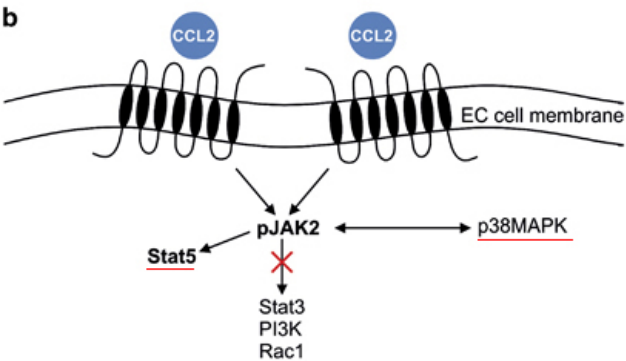
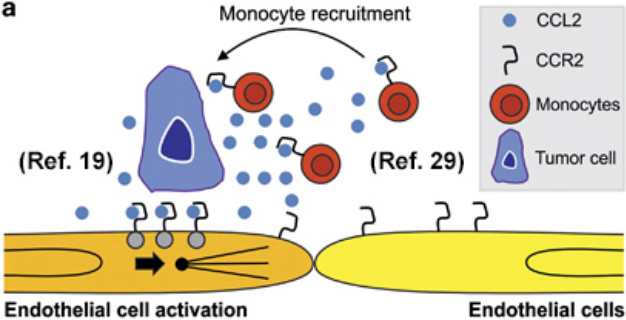
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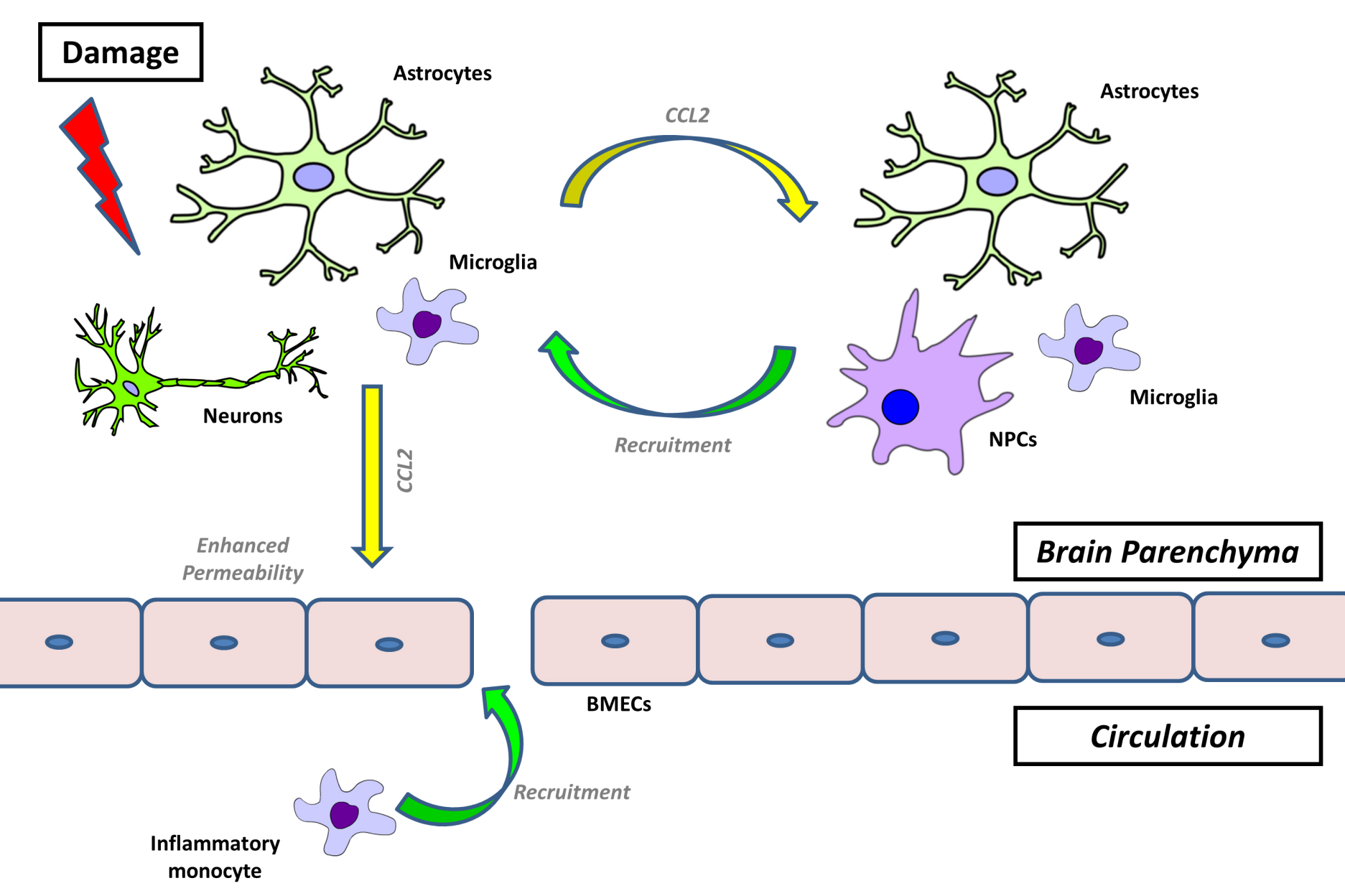
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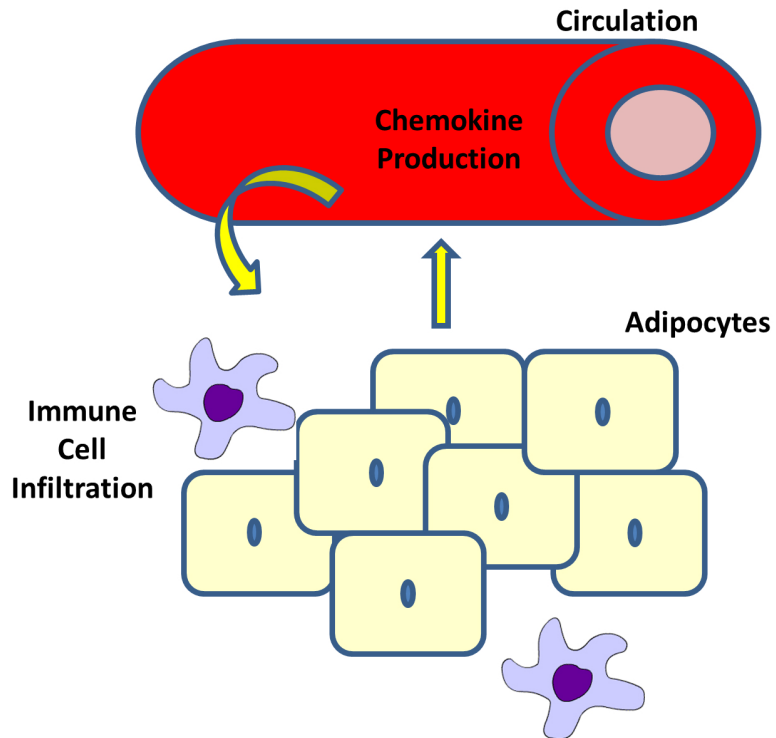
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